Functional genomics: **The worm scores a knockout**

Stuart K. Kim

Following the completion of the genome sequence of *Caenorhabditis elegans*, four independent studies have now assessed the functions of more than a third of the worm's genes by analysing the phenotypes caused when each of a large set of genes is inactivated by RNA interference.

Address: Departments of Developmental Biology and Genetics, Stanford University, Stanford, California 94305, USA.
E-mail: kim@cmgm.stanford.edu

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The success of the various genome projects has provided enormous resources for studying biological processes on a grand scale. Among metazoa, the complete sequence of the nematode *Caenorhabditis elegans* was reported in 1998 [1], followed by that of the fruitfly *Drosophila* [2] in 2000; the human genome sequence has been completed and will be published early this year. With the DNA sequence completed, we are confronted with the enormous challenge of uncovering the functions of the genes defined by the genome projects. The magnitude of this challenge is enormous: in *C. elegans*, so far only 1572 genes have been studied at the biochemical or genetic levels, out of a total of 18,972 genes — just 8% of the genome [3]. It is clear that we need to use functional genomics approaches to address gene functions on a global scale.

Now four papers, two in *Current Biology* [4,5] — one [5] elsewhere in this issue — and two in *Nature* [6,7], have reported important advances in *C. elegans* functional genomics. These studies represent the largest attempt so far to document the mutant phenotypes of many genes in parallel in a given species. All four groups used the technique of double-stranded (ds)RNA-mediated interference (RNAi) to determine the loss-of-function phenotype of genes comprising in total about one-third of the genome. Genetic screens using RNAi have an advantage over conventional mutagenesis approaches, as the genetic map position and molecular nature of the gene are already known. These data will be an incredibly useful resource for those working directly on *C. elegans*. And as basic gene functions are often conserved, the data will also be informative for those working on related genes in other species.

The technique that all four groups used, RNAi, is based on a fascinating biological phenomenon [8]. In many species, expression of a particular dsRNA sequence has been shown to cause the specific degradation of the corresponding mRNA, resulting in a nearly complete loss-of-function phenotype. Thus, one can use RNAi to efficiently find mutant phenotypes for genes defined by sequence, without having to identify mutations in the gene itself, greatly speeding up analysis of mutant phenotypes. RNAi works exceptionally well in *C. elegans*, and the dsRNA can be delivered by injection, by soaking worms in a solution of dsRNA, or by feeding worms bacteria expressing dsRNA. Delivering dsRNA by feeding is particularly useful, because it is less labor intensive than microinjection and could thus be used by many labs to subsequently repeat the whole-genome RNAi screen to search for particular RNAi phenotypes.

The four studies [4–7] illustrate the power of RNAi on a whole genome level. Hyman's group [6] systematically analyzed the RNAi phenotype of nearly all of the genes on *C. elegans* chromosome III — 2,174 genes, 96% of the genes on chromosome III. They designed polymerase chain reaction (PCR) primers to amplify genomic regions corresponding to each gene on chromosome III, prepared dsRNA from the PCR products and injected the dsRNAs into worms. They then observed visible phenotypes using a dissecting microscope, and cellular phenotypes using time-lapse differential interference contrast (DIC) microscopy. As in all four studies, there was good agreement between results obtained using RNAi and previously reported germ-line and embryonic phenotypes (20/24 genes), but only moderate agreement for previously reported larval or adult phenotypes (9/31 genes). The experiment revealed 281 genes (12.9%) with an RNAi phenotype, including 133 with a cellular phenotype. These 133 genes include those involved in meiosis, nuclear appearance, cell division, the pace of development and embryonic morphology.

In order to enrich for genes that function in the germ line or in embryogenesis, the Kemphues group [4] prepared an oocyte cDNA library. They used RNAi on 350 different oocyte cDNAs, and found a set of 81 non-redundant cDNA clones with essential roles in embryogenesis. In a similar manner to Hyman's group, Kemphues and colleagues used time-lapse recordings of early embryogenesis, which revealed cellular defects in relatively high detail. The high resolution provided by time-lapse recording of individual embryonic cells is important, because the level of detail in many cases suggests which specific cell or developmental events are defective in the RNAi-treated embryos.

Sugimoto's group [5], whose paper appears elsewhere in this issue, examined the RNAi phenotype of 2,479 genes

Table 1

Summary of the results of the four recent *C. elegans* functional genomics studies.

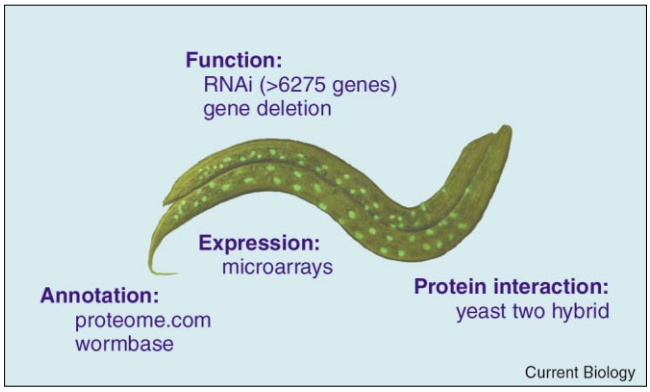
Group	RNAi method	Genes	No. genes analyzed	No. genes with phenotypes
Hyman [6]	Injection	Chromosome III	2,174	281
Kemphues [4]	Injection	Oocyte cDNAs	350*	81
Sugimoto [5]	Soaking	ESTs	2,479	675
Ahringer [7]	Feeding dsRNA	Chromosome I	2,416	339
Total			> 6,275 (33%)†	> 888†

*Number of cDNA clones, some of which represent identical genes. †These totals do not include those from Piano *et al.* [4].

defined by non-redundant cDNA clones. They prepared dsRNA from each of the cDNA clones, soaked worms in the dsRNA, and then scored the rough phenotype — such as embryonic or larval lethal — using a dissecting microscope. They found 675 genes (27%) showing a gross phenotype at the level of the dissecting microscope.

Finally, Ahringer's group [7] focused on genes from chromosome I. A key advantage of the approach used by this group is that they induced RNAi by feeding bacteria expressing dsRNA to worms. They prepared PCR fragments corresponding to genes on chromosome I, cloned each into an expression vector for dsRNA, and produced a library of bacterial strains corresponding to 2,416 genes from chromosome I (87%). They fed these dsRNA-producing bacteria to worms, scored a wide range of phenotypes using a dissecting scope, and found a total of 339 genes (13.9%) with clear phenotypes. An important aspect of this experimental approach is that, once made, the library of dsRNA-producing bacteria can be used again by other researchers in subsequent screens for particular RNAi phenotypes.

Figure 1



An illustration of the kinds of functional genomic approaches being taken with *C. elegans*. Data from each of these approaches can be found at these web sites: function, http://mpi-web.embl-heidelberg.de/dbScreen/, www.wormbase.org, http://watson.genes.nig.ac.jp/db/rnai_s/index.html, www.rnai.org and http://elegans.bcgsc.bc.ca/knockout.shtml; annotation, http://www.proteome.com/databases/ and http://www.wormbase.org/; expression, http://cmgm.stanford.edu/~kimlab/wmdirectorybig.html and ftp://spliceome.org/pub/Chip_Data; protein interaction, http://cancerbiology.dfci.harvard.edu/cancerbiology/ResLabs/Vidal/

The four groups collectively analyzed RNAi phenotypes from more than 6275 genes — 33% of the genome — and found phenotypes for more than 888 genes (Table 1). This is a significant accomplishment. The work described here adds to the mutant phenotypes being assembled by the *C. elegans* knockout consortium. In most cases, the phenotypes of these genes were not previously known; for example, in the work by Ahringer and colleagues [7], the 339 genes with phenotypes included 308 in genes for which no phenotype had previously been described. Previously, there was a total of 1572 *C. elegans* genes in which both the DNA sequence and mutant phenotypes were known [3]. Many of these genes have subtle mutant phenotypes, such as defective osmotic avoidance or chemosensation, such that only about 1173 genes have a mutant phenotype that would have been detected in these four RNAi studies ([3]; P. Gonczy, personal communication). Hence, the RNAi screens identified about 56% as many genes as had been previously known, or approximately 76% as many genes including only those with phenotypes that would have been scored in the RNAi projects.

These four global RNAi studies have provided functional information about a significant portion of the genome, albeit at a shallow level. Although the time-lapse video recordings performed by the Hyman [6] and Kemphues [4] groups documented the cellular RNAi phenotypes in reasonable detail, the mutant phenotypes for each of the genes could be studied in far greater detail, including analysis using molecular markers and the isolation of genetic mutations. There is great excitement and anticipation that the remaining two thirds of the genes in the *C. elegans* genome will soon be scanned using RNAi, achieving a milestone in the '*C. elegans* functional genome project'. Now that they have worked out the experimental approach for the first third of the genome, the *C. elegans* groups seem well poised to finish most of the remainder of the genome in relatively short order.

Functional genomic studies in *C. elegans* are advancing rapidly along a number of different and interconnected fronts. Along with the RNAi work discussed here, gene functions are being determined by annotation of the genome, by expression analysis using DNA microarrays and by finding protein-binding interactions using a global yeast two-hybrid approach (Figure 1) [3,9–13]. Together, these approaches will deliver information on the basic properties of gene function: sequence, mutant phenotype, expression profile and protein-binding interaction. These approaches make *C. elegans* an attractive model organism for genetics and functional genomics, serving not only those that already work on *C. elegans* but also those that work on genes in other organisms with a function conserved in *C. elegans*.

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